Laser Doppler Flowmetry in CA1 Sector of Hippocampus and Cortex After Transient Forebrain Ischemia in Gerbils

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Background and Purpose: Local differences in the hemodynamic response to transient ischemia could be involved in the development of selective vulnerability. These differences were studied in vulnerable and nonvulnerable regions of the brain.

Methods: Five gerbils were subjected to 10 minutes of bilateral forebrain ischemia, and cerebral blood flow was measured continuously in the frontal cortex and CA1 sector of the hippocampus using laser Doppler flowmetry. Carotid artery pressure was recorded simultaneously with a pressure transducer.

Results: After induction of ischemia, blood flow in the cortex and CA1 sector decreased to 11.8% and 18.0% of the baseline value, respectively. After release of the vascular occlusion, blood flow in the cortex returned to the preischemic level at 7.5 minutes (recovery time), reached the hyperemic peak (123.8%) at 12.4 minutes (peak latency), and again decreased to the preischemic level at 27.2 minutes. In the CA1 sector, blood flow returned to the preischemic level at 2.1 minutes, reached the hyperemic peak (122.2%) at 5.7 minutes, and decreased again to the preischemic level at 21.3 minutes. In both the cortex and CA1 sector, recovery time and peak latency correlated inversely with the amount of residual blood flow during ischemia. Histologically, cortical neurons were not injured but only 14.6% of CA1 neurons survived 1 week after ischemia.

Conclusions: CA1 neurons were selectively injured despite the milder percentage decrease of blood flow during ischemia and the more prompt recovery of flow after ischemia. These findings stress the importance of intrinsic rather than hemodynamic factors in the pathogenesis of selective vulnerability of CA1 neurons after transient bilateral forebrain ischemia. (Stroke 1992;23:1349-1354)

KEY WORDS • cerebral blood flow • cerebral ischemia • ultrasonics • gerbils

Neurons of the CA1 sector of the hippocampus are selectively vulnerable to brief periods of global ischemia. This phenomenon has received considerable attention in the past, and numerous studies have attempted to elucidate the underlying mechanisms using various animal models of cerebral ischemia.

Two basically different pathophysiological processes, namely, pathoclitic (intrinsic) and hemodynamic (extrinsic) factors, have been discussed as playing the dominant role in the development of selective vulnerability. Vogt and Vogt originally emphasized the importance of inherent morphological or biochemical properties for the “pathoclitis” of selectively vulnerable neurons. Since then many studies have focused on various intrinsic factors such as anatomical connections, neurotransmitters, and receptor density, and there is now substantial evidence that glutamate-mediated excitation, disturbances of intracellular calcium homeostasis, and inhibition of protein synthesis are of particular importance.

The role of hemodynamic (extrinsic) factors has also been stressed since Spielmeyer first noted a difference in the vascular supply of selectively vulnerable and resistant brain regions. It is generally held that selective vulnerability is not caused by a more severe density of ischemia, but there are uncertainties about the role of postischemic hemodynamic factors, which are known to affect postischemic recovery. Postischemic recruitment of capillaries is less marked in the CA1 sector than in the cortex, but postischemic hyperemia, measured by quantitative autoradiography, is more pronounced.

For these reasons, we became interested in studying the differences in ischemic and postischemic hemodynamics between selectively vulnerable and tolerant brain regions. We report the results of continuous, simultaneous laser Doppler flow measurements in the vulnerable CA1 sector of the hippocampus and in the resistant cortex of gerbils subjected to 10 minutes of transient forebrain ischemia.

Materials and Methods
Twenty adult Mongolian gerbils (Meriones unguiculatus) of either sex weighing 50–80 g were used. Before the experiment, the animals had free access to water and food. Atropine sulfate (0.01 mg/g body wt) was injected subcutaneously approximately 10 minutes be-
fore anesthesia. Anesthesia was induced with 3% halothane and continued with 1% halothane in a gas mixture of 70% nitrogen and 30% oxygen. Rectal temperature was monitored and kept close to 37.5°C with a feedback-controlled heating system (LM-60, List Medical Electronic, Darmstadt, FRG). Cranial temperature was measured with a needle-type thermistor electrode (type 513 hypodermic temperature probe, YSI Inc., Yellow Springs, Ohio) inserted under the parietal scalp. Both common carotid arteries were exposed, and a 5-0 nylon thread was looped around each artery and connected to manipulators for production of arterial occlusion by retraction of the thread. The left external carotid artery was exposed under an operating microscope, and a 27-gauge needle connected to a pressure transducer and a microinfusion pump was inserted with its tip close to the carotid bifurcation.10 Carotid artery pressure was monitored throughout the experiment, clotting in the needle being prevented by the infusion of saline at 2–5 μl/min.

After stabilization of the carotid artery pressure, the left frontoparietal cortex was exposed and a corticostomy approximately 1–1.5 mm in diameter was made 1.5 mm posterior to the bregma and 2 mm left of the sagittal suture to expose the floor of the lateral ventricle at the roof of the hippocampus CA1 sector. To this purpose the arachnoid membrane was incised and the cortex was carefully suctioned using a surgical vacuum pump. Exposure of the hippocampus was confirmed by observation of the lateral ventricular floor and leakage of a small amount of cerebrospinal fluid.

A laser Doppler probe (PF303, Perimed, Stockholm, Sweden) was then placed stereotaxically over the lateral ventricular floor, and another probe of the same type (PF303) was placed on the frontal cortex 1–2 mm anterior to the coronal suture and 2 mm lateral to the midline. Both sites were inspected microscopically to ascertain the absence of large vessels. Artificial cerebrospinal fluid warmed by a heating system to 37.5°C was used to irrigate the sites of probe placement throughout the experiment, and blood flow was measured continuously by laser Doppler flowmetry (Periflux Pf2, Perimed). Flow returned to zero after killing the animal with an overdose of anesthesia in a separate experiment using the same measuring unit. Bilateral transient forebrain ischemia was induced by occluding both common carotid arteries for 10 minutes and was discontinued by removing the threads. Blood flow and carotid artery pressure were monitored before, during, and after recirculation for 1 hour. In group 1 (n=6), the gerbils were killed with an overdose of pentobarbital (100 mg/kg) after this interval. In group 2 (n=6) the animals were weaned from anesthesia, returned to their cages, and kept alive for 1 week before being killed with an overdose of pentobarbital. The brains were then removed and fixed by immersion in 4% and 10% buffered formalin for 1 and 4 days, respectively. Coronal sections 5 μm thick taken at the center of the laser Doppler flow probe placement sites on the frontal cortex and lateral ventricular floor were stained with Luxol fast blue and cresyl violet and with hematoxylin and eosin. In group 2 the number of intact neurons in the hippocampus CA1 sector was counted and expressed as cells per millimeter of stratum pyramidale.

Nonischemic control animals (n=8) were treated in a similar way except for vascular occlusion. Four control gerbils were killed at 1 hour and the other four at 1 week after sham operation.

All values are expressed as mean±SD. Statistical evaluation was performed using Bartlett's test for analysis of variance, unpaired Student's t test for comparison of experimental and control groups, and paired Student’s t test for comparison of paired data obtained in each animal.

**Results**

**Control Groups**

Rectal temperature was kept close to 37.5°C and cranial temperature at about 37.0°C during anesthesia. Recordings of laser Doppler blood flow from the frontal cortex and hippocampus were stable except for a slight but nonsignificant decrease during sham operation (manipulation of the carotid arteries). Mean carotid artery pressure was 71.3±9.6 mm Hg and stable.

No tissue injury was observed in the area of flow measurement in the frontal cortex. A defect of the cortex approximately 1.5 mm wide and continuing to the lateral ventricle over the hippocampus CA1 sector was observed in the section at the corticostomy site. In the 1-hour survival group, the wall of the tissue defect was mildly edematous and occasionally slightly hemorrhagic. The density of pyramidal neurons in the hippocampus CA1 sector 1 week after the sham operation was 244.5±18.4/mm.

**Experimental Groups**

Rectal temperature in the experimental groups was stable and similar to that in the control groups. Cranial temperature decreased during ischemia by approximately 0.3°C but returned promptly to the preischemic level after recirculation and remained at this level throughout the experiment.

Typical recordings of laser Doppler blood flow and carotid artery pressure are shown in Figure 1. Carotid artery pressure was 64.7±8.5 mm Hg before occlusion of the carotid artery (Figure 2). After occlusion, carotid artery pressure decreased promptly to 11.0±5.0 (range, 4–16) mm Hg and remained at this level during ischemia. After release of the vascular occlusion, carotid artery pressure returned promptly to the preischemic level after recirculation and remained at this level throughout the experiment.

Blood flow of the cortex and hippocampus CA1 sector decreased after vascular occlusion and remained constant throughout the 10 minutes of ischemia (Figure 3). Residual flow in the CA1 sector of the hippocampus was consistently higher (18.0±7.3%) than in the cortex (11.8±4.8%), and the difference was significant (p<0.05). After release of the vascular occlusion, return of blood flow to the baseline level was delayed for 2 minutes, the pressure after 1 minute of recirculation being 60% of the preischemic level.
First, residual blood flow and carotid artery pressure were significantly correlated in both the hippocampus CA1 sector and the cortex (Figure 4). During ischemia, residual blood flow and carotid artery pressure were significantly correlated in both the hippocampus CA1 sector and the cortex (Figure 4). After ischemia, recovery time and latency of the postischemic hyperemic peak were inversely correlated with residual blood flow in both regions (Figure 5). The quality of postischemic recirculation consequently depended on the depth of ischemia which, in turn, was a function of carotid artery pressure.

Histologic findings after 1 hour of recirculation (group 1) were essentially the same as in the control group. No ischemic injury was detectable in either the frontal cortex or the hippocampus CA1 sector (Figure 6A). One week after ischemia (group 2) the frontal cortex was still intact, but there was a significant loss of pyramidal neurons in the CA1 sector at the level of the corticostomy (Figure 6B). The density of remaining pyramidal neurons was only 35.8±33.5/mm.

Discussion

Laser Doppler flowmetry is based on measurement of the number and mean velocity of red blood cells in microvessels, using the coherent properties of laser illumination. Recently, the validity of this technique was confirmed for measurement of the cerebral microcirculation under physiological and pathological conditions by demonstrating a close correlation with blood flow values obtained by the hydrogen clearance or microsphere techniques.

The sensitive volume of laser Doppler flowmetry is less than 1 mm, and the main signal received from tissue originates from a depth of less than 1 mm. Thus, the tissue recorded by placing the probe on the surface of the frontal cortex is gray matter and that by placing the probe on the lateral ventricular floor is the hippocampus CA1 sector because this sector is located under the ventricular surface (Figure 6). To eliminate erratic flow recordings, probes were not placed in the vicinity of vessels more than 200 μm in diameter.

Flow recording from the hippocampus CA1 sector required production of a small corticostomy approximately 1.5 mm in diameter. In rats subjected to transient global ischemia, interruption of neuroexcitatory input to the CA1 sector by lesioning the entorhinal cortex, perforant path, or fimbria/fornix region together with the overlying cortex significantly ameliorated CA1 injury. To examine the possibility that the corticostomy in the present experiment reduced the vulnerability of the CA1 sector, histological examination was carried out 1 week after the intervention. Only 15% of CA1 neurons survived, showing that the corticostomy did not prevent neuronal death despite the possible reduction in excitatory input.

The main results of the present study are 1) residual blood flow after carotid artery occlusion varied considerably among gerbils but was consistently higher in the hippocampus CA1 sector than in the cortex, 2) reflow after removal of the vascular clips was consistently delayed in both the cortex and CA1 sector and was closely correlated with the severity of ischemia, and 3) the recirculation delay and peak latency of hyperemia were significantly shorter in the CA1 sector than in the cortex.
FIGURE 3. Plots of mean±SD laser Doppler blood flow in left frontal cortex (left) and hippocampus CA1 sector (right) in control gerbils (upper) and in gerbils subjected to 10 minutes of bilateral common carotid artery occlusion (lower). Note faster return of blood flow after ischemia in CA1 sector.

FIGURE 4. Scatterplot of correlation between carotid artery pressure and laser Doppler blood flow in hippocampus CA1 sector (○) and cortex (●) during 10 minutes of bilateral common carotid artery occlusion in gerbil. Parameters correlated significantly in both hippocampus CA1 sector and cortex. Note that blood flow was always higher in hippocampus CA1 sector than in cortex. Laser Doppler blood flow is expressed as percent of preischemic baseline level.

hippocampus although only the hippocampus suffered ischemic injury.

In rat models of forebrain ischemia induced by four-vessel occlusion or bilateral carotid artery occlusion with systemic hypotension, blood flow has been reported to be uniformly near zero in all cerebral structures. In gerbils, in contrast, significant residual blood flow may persist after vascular occlusion. This residual flow differs in different parts of the brain. After bilateral common carotid artery occlusion it amounted to 5.0±1.3 ml/100 g/min in the cortex and 8.0±2.7 ml/100 g/min in the hippocampus. In the present study residual flow was 11.8±4.8% (6-17%) of the baseline level in the cortex and 18.0±7.3% (10-27%) in the hippocampus CA1 sector. The greater distance of cortical arterioles from the circle of Willis may explain the observed lower residual flow during ischemia in the cortex. Ours and the previous studies, therefore, clearly demonstrate that ischemia is less severe in the hippocampus. Selective damage of the CA1 sector, therefore, cannot be related to a more severe ischemic impact.

However, ischemic injury is also dependent on the quality of postischemic recirculation. In the present experiment a period of impaired reflow was consistently observed after removal of the vascular clips in both the hippocampus CA1 sector and the cortex, although the duration varied widely from 30 seconds to 13.2 minutes. Our findings are in line with those of several other studies that also reported transient no-reflow or impaired reflow after brief periods of cerebral ischemia. Other studies did not show impairment of reperfusion, but these studies may have overlooked transient recirculation deficits because blood flow was measured intermittently.

Major factors influencing the severity of no-reflow are duration of ischemia and postischemic brain perfusion pressure. The present study confirms that reperfusion impairment increases with decreasing residual blood flow, which is in line with the contention that viscosity of the blood rises with declining flow rate.

The role of impaired reflow on the manifestation of ischemic tissue injury is still unclear. Obviously, persisting no-reflow results in cell death. However, impairment of postischemic recovery has also been documented after transient disturbances of reflow. In the present study the frontal cortex, which did not suffer ischemic injury, exhibited greater impairment of reflow than the CA1 sector of the hippocampus, in which severe neuronal injury developed. Thus selective vulnerability of CA1 neurons cannot be explained by impaired reflow.
Another hemodynamic factor that could be of importance for the manifestation of ischemic injury is postischemic reactive hyperemia. Reactive hyperemia develops after global or focal brain ischemia as soon as the early postischemic reperfusion impairment has been resolved. We observed that both the hippocampus CA1 sector and the cortex showed the same degree of postischemic hyperemia, but peak latency was significantly shorter in the CA1 sector, where hyperemia reached its peak at 5.7 minutes compared with 12.4 minutes in the cortex. This difference could be of significance for the development of postischemic brain edema which, however, has been discussed controversially in the past. After 3 hours of focal ischemia hyperemia accentuated brain edema, but after 1 hour of global ischemia it did not. A likely explanation for this discrepancy is the permeability of the blood–brain barrier, which increases after 3 hours but not after 1 hour of ischemia. In the present investigation the blood–brain barrier remained presumably intact because the duration of ischemia was only 10 minutes. It is, therefore, unlikely that hyperemia induced or aggravated postischemic brain edema.

Finally, the possibility of postischemic hypoperfusion as a pathogenetic factor should be considered. Consistently developing after reactive hyperemia has ceased, postischemic hypoperfusion may last for several hours into the recirculation period. In the present investigation of the first hour of recirculation, postischemic hypoperfusion appeared slightly earlier in the CA1 sector than in the cortex, but the severity of hypoperfusion in the two structures was the same. Later differences in blood flow between vulnerable and nonvulnerable regions may exist but have not been demonstrated up to now. It is conceivable that the earlier appearance of hypoperfusion in the hippocampus is responsible for the fact that recruitment of capillaries in the CA1 sector is less marked than in the cortex. However, the rather small difference in the time of onset of hypoperfusion in vulnerable and nonvulnerable regions is difficult to reconcile with the pronounced difference in histological injury.

In conclusion, the present study of changes in blood flow during and after 10 minutes of bilateral common carotid artery occlusion in gerbils does not support the contention that selective vulnerability of the CA1 sector of the hippocampus is related to ischemic or postischemic hemodynamic disturbances. It is, therefore, more likely that this type of injury is the result of intrinsic "pathoclitic" particularities that differentiate selectively vulnerable neurons from other neuronal populations of the brain.

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The phenomenon of selective vulnerability of the CA1 sector of the hippocampus to ischemia has been known for several years. While it is not necessarily believed that differences in ischemic or post-ischemic blood flow account for the selective vulnerability, the discontinuous nature of previously used blood flow measurement techniques has not totally eliminated this possibility. The study by Kuroiwa et al has used a continuous indicator of blood flow, laser Doppler flowmetry, to further enlighten this question. Using two laser Doppler probes, they have provided continuous, simultaneous measurements of changes in blood flow in the hippocampus and cortex of ischemic and postischemic gerbil brains. The results show quite convincingly that flow differences in the 1-hour post-ischemic period are not the cause of the selective vulnerability of the hippocampus. The authors are to be commended on their creative use of a new technique to clarify an old problem. Their results further support the importance of other, intrinsic "pathologic" differences in the pathogenesis of selective hippocampal cell death.

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